ON QUININE IN ANIMAL TISSUES AND LIQUIDS, WITH METHODS FOR ITS ESTIMATION

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INTRODUCTION

Our main objects in the work recorded below have been:—

1. To devise methods delicate enough for the estimation of quinine in the various organs, tissues and liquids of the animal body.

2. To apply these methods and obtain information concerning the quinine-content of the blood, urine, and tissues, and the metabolism of quinine in the body in the hope that light would thereby be thrown on the problems of blackwater fever, quinine intoxication, and the therapeusis of malaria.

Many previous observers have investigated the elimination of quinine in urine and faeces, and the more important results of their labours may be briefly stated as:—

(i) proof that of the quinine administered, whether by mouth or by intravenous or intramuscular injection, only a fraction, varying with different patients from 23 to 66 per cent., reappears in the urine;

(ii) proof that, except when given in some insoluble form such as the tannate, any elimination of quinine by the faeces is ordinarily so slight as to be negligible;

(iii) evidence that no recognizable derivative of quinine is excreted in the urine;

(iv) evidence that quinine administered in solution by the mouth is absorbed with great rapidity, and appears in the urine almost as rapidly as when it is administered intravenously.

For an adequate understanding of the methods described below, reference to a paper recently published by two of us (8) describing a method for the extraction and nephelometric estimation of the minute amounts of quinine occurring clinically in human blood and urine is essential.

METHOD OF ESTIMATION APPLICABLE TO MOST TISSUES (AMMONIUM SULPHATE METHOD)

About 5 grams of the tissue is transferred direct from the animal into a weighed flask containing about 10 c.c. of a saturated aqueous solution of ammonium sulphate and 0·6 per cent. sulphuric acid. The flask is re-weighed, then boiled for two minutes and its contents filtered under pressure through a Gooch crucible or Buchner filter. The residue is then pulped in a mortar with glass-powder and again boiled up in the original flask with successive lots of acidulated ammonium sulphate solution. The combined filtrates are shaken with ether to extract oily matters, then alkalised strongly with ammonia and again shaken up with ether to extract the quinine. The quinine obtained is estimated nephelometrically exactly as in the method for blood.

The method has been laboriously tested with each of the tissues referred to in the following table. The tissues were first reduced to a pulp. About 5 grams of the pulp were then mixed thoroughly with a measured volume of a solution of quinine hydrochloride. In many cases the mixture stood half an hour or more before it was boiled with acidulated ammonium sulphate solution. The observers did not know the correct results beforehand.

CONTROL ESTIMATIONS WITH THE AMMONIUM SULPHATE METHOD.

Tissue	Animal	Mgms. Q. Added	Found	Error %
Spleen	Guinea-pig	0·05	0·049	− 2·5
,,	,,	0·04	0·0385	− 4
Kidney	,,	0·048	0·045	− 4·8
,,	,,	0·05	0·048	− 5
Suprarenal	,,	0·05	0·048	− 5
,,	,,	0·04	0·0385	− 4
Muscle	,,	0·05	0·0476	− 4·8
Liver	,,	0·05	0·04	− 20
,,	,,	0·05	0·0384	− 23
,,	,,	0·05	0·0435	− 13
Brain	Sheep	0·10	0·05	− 50
,	,,	0·05	0·35	− 30
,,	,,	0·10	0·05	− 50

Qualitative control experiments have been made in each case and also with bone-marrow and beef-suet. In the absence of quinine, an absolutely negative Tanret turbidity test was found invariably.

The results have been, as shown by the table, invariably deficits. With none of the tissues examined, however, except liver and brain, have these deficits exceeded 5 per cent. of the minute total, and we believe them to have been due within this limit largely to losses in manipulation of the ether used for extracting the quinine.

It is probable that a simple method which is capable of giving results like the above in an average time of one and a half hours, or some slight modification of it adapted for the particular alkaloid sought, would be found very useful in medico-legal and pharmacological work.

With brain and liver, the deficits ranged from 13 per cent. to 50 per cent., and it appeared at first probable either that boiling the tissues with saturated Am_2SO_4 solution failed to extract the whole of the alkaloid, or that some other substance which diminished the Tanret turbidity was extracted with it.

Various alcohol-extraction methods were accordingly devised, and after many failures, the following procedure was found to give accurate results in the case of brain and fat, but in the case of liver deficits even greater than those found by the first process. We have not tested it with faeces.

ALCOHOL EXTRACTION METHOD

Weigh out about 10 grams of tissue, grind up to a fine pulp with powdered glass and transfer it to a flask, with the aid of boiling absolute alcohol. Boil and filter into a graduated cylinder. Repeat the boiling and filtering with three further lots of alcohol. Note the volume of filtrate, pour it into five times its volume of 1 per cent. sulphuric acid and shake thoroughly for five minutes. Extract its fats by shaking with three successive lots of pure ether. For every 100 c.c. of the fat-free liquid add 5 c.c. of 25 per cent. lead acetate solution and filter off an aliquot portion of the whole into a stoppered cylinder. Saturate it with ammonium sulphate, pipette off the layer of alcohol-ether which separates, and again extract with ether until the extracts are colourless. Alkalise with ammonia and extract the quinine with four successive lots of ether, evaporating each lot as it separates in one of the tubes gauged for nephelometry. Dissolve the residual quinine by boiling it with a sufficiency of a known volume of saturated aqueous ammonium sulphate, and estimate it nephelometrically.

CONTROL ESTIMATES BY THE ALCOHOL-EXTRACTION METHOD.

Tissue	Animal	Mgms. Q. added	Found	Error %
Brain	Sheep	0·10	0·095	− 5
,,	,,	0·025	0·0254	+ 1·6
,,	,,	0·05	0·048	− 4
Liver	Guinea-pig	0·10	0·066	− 33
,,	,,	0·05	0·0385	− 23
Liver	Ox	0·10	0·03	− 66
,,	,,	0·10	0·04	− 60
,,	,,	0·059	0·0286	− 51

It was evident from the good results with brain that the large deficits found by the alcohol method when applied to liver were not due to incomplete extraction of quinine.

Trying the ammonium sulphate method again, but with the variation that the liver pulp was boiled *before* the quinine was added, it was found to give results as accurate as those obtained with spleen, kidney, suprarenal, etc. This finding made the idea of an interfering substance in liver which diminished Tanret turbidity a very improbable one, and forced us to contemplate the possibility that the liver rapidly destroyed quinine or in some way modified it even post-mortem.

The experiments recorded on pp. 230-233 below show the interesting fact that this is actually the case, and also that the quinine is altered under conditions which preclude general protoplasmic activity. They show further that this rapid alteration is the sole cause of the large deficits obtained by the ammonium sulphate process when applied to liver, and that when the liver pulp was 'inactivated' at once after adding the quinine by saturating it with ammonium sulphate the method gave excellent results, results, *vide* p. 232, Exp. E.

Pending definite knowledge whether other tissues* differ from liver only in the rapidity of their action on quinine or whether they are entirely inactive, the precaution of boiling all the tissues as soon as possible is clearly advisable.

DETECTION OF QUININE IN FAECES

Grind about 10 grams of faeces with enough 1 per cent. sulphuric acid to make a fine suspension, acid in reaction, and transfer it to a beaker. Add ammonium sulphate till saturated, boil for a few minutes and filter under suction through a Buchner funnel. For every 10 c.c. of filtrate add 0·5 c.c. of 25 per cent. lead acetate solution and filter—the precipitated lead salts carry down with them some substance which would otherwise appear in the final product and give Tanret turbidity. Shake up the acid filtrate with three successive 8 c.c. lots of purified ether to remove 'oily matters'; then alkalise with ammonia and shake up with three further lots of ether to extract the quinine. To the residue left when the ether is

* We have recently ascertained that guinea-pig's muscle destroys quinine under similar conditions.

evaporated off, apply either the Herapathite or the Tanret turbidity test. The above procedure easily detects 0·01 mgm. of quinine in 10 grams of faeces. It does not, however, give correct quantitative results by the nephelometric method—the amounts found are usually about 60 per cent. of the amount added, and must therefore be regarded as minimal.

RESISTANCE OF QUININE TO CHANGE IN PUTRIFYING FAECES AND URINE

We have not ascertained the cause of the deficit found in the nephelometric estimate of quinine in faeces. If we may judge by a single experiment, it is not due to destruction of quinine by faecal bacteria. To 100 c.c. of a thin cream of faeces rubbed up with water, 5 mgms. of quinine were added in feebly acid solution. Immediate estimation gave 3·1 mgms. Estimation after three days' incubation at 37°C. gave 3·15 mgms. Typical Herapathite crystals were obtained.

Similarly with 250 c.c. of putrifying urine to which 0·625 mgm. of quinine were added. Samples, each of 50 c.c., were taken at intervals. The reaction remained acid throughout. Estimated at once, 97 per cent. was found; after 2, 8, and 31 days respectively, 98 per cent., 96 per cent. and 98 per cent. were found.

In another urine after eight days alkaline putrification at laboratory temperature the amount found was exactly equal to that originally added.

ESTIMATE OF QUININE IN BILE

The bile is first of all 'defaecated' precisely as if it were so much urine (8). An aliquot part of the filtrate is then treated by the ammonium sulphate method described for blood and the extracted quinine is estimated nephelometrically.

	Mgms. of quinine given	Mgm. found	Error %
Ox bile (5 c.c.)	0·04	0·03846	− 4·0
,, ,,	0·20	0·1925	− 4·0

DETECTION OF QUININE IN SPUTUM, GASTRIC CONTENTS OR MILK

Treat exactly as if so much blood. Control experiments have shown that in the absence of quinine the residue finally obtained gives not a trace of Tanret turbidity. We have not made quantitative estimations, but have little doubt that the method would give good results.

ADDENDA ON THE ESTIMATION OF QUININE IN BLOOD

We have had extensive experience of the nephelometric method recently published by two of us, and the following additional instructions and comments should be found useful:—

1. Great care must be taken to dissolve in the saturated Am_2SO_4 solution every trace of the extracted quinine To do so without risk of altering the quinine by excessive heat, it is well to heat the test-tube and its contents in a brine bath.

2. Differences of as little as 2 per cent. in the quinine-content of two solutions can be detected with certainty by means of the adjustable-slit nephelometer devised by one of us (W. R.) provided the matching tubes are of exactly equal calibre and the observer is not fatigued. We find it best to make the whole series of matching tubes ourselves out of a single piece of 'quill' glass tubing, 13 mm. in diameter, along which a closely-fitting cork can be moved with equal friction throughout its entire length.

3. We find it advantageous to place all the matching tubes with their turbid contents in a bath of boiling brine or saturated ammonium sulphate for a few minutes until they become clear. Then cool equally in running water for ten minutes. Perfect mixing is secured, the turbidity reappears unimpaired, and any effects due to original differences in the time and rate of mixing with Tanret's reagent are completely eliminated. This treatment cannot be repeated more than once without risk of introducing error.

4. When the total quinine is not more than 0·01 mgm., 2 c.c. of saturated sulphate solution (instead of 5 c.c.) should be used to dissolve it, and a correspondingly reduced volume of Tanret's reagent added. In 13 mm. test-tubes the height of the column amply suffices for the nephelometer, and the greater concentration of the quinine permits 0·005 mgm. to be estimated.

5. We have ascertained for the benefit of workers in hot climates that for the estimation of quinine in blood medicinal chloroform may, without any loss of accuracy, be substituted for the ether. This is not true for urine.

ACTION OF LIVER ON QUININE

The selected experiments recorded below supply further detail concerning the conversion of quinine effected by the liver. The rate of change is at first rapid, but soon slows down. When it is complete, the residue of an ether extract of the alkalised liver preparation gives not a trace of Tanret turbidity; the product is therefore presumably not alkaloidal, and is certainly not quinotoxin. What it is our experiments do not show. They have been directed to ascertain first of all what conditions as regards antiseptics, reaction, and oxygen supply are favourable, and how the active agent or agents can be obtained free from gross accompaniments.

The change produced in the quinine in all probability represents, in part at least, that which takes place in the 23 to 93 per cent. (*vide* p. 251) of the ingested quinine which disappears in the human body—apparently without any abnormal substance appearing in the urine. Since nothing whatever is known about the metabolites of quinine in man or animals and it is conceivable that one or other of them is actively therapeutic in malaria or concerned, directly or indirectly, with the genesis of blackwater fever, a study of 'hepatized quinine' may have practical importance in medicine as well as chemical and physiological interest.

Owing to the slower rate of conversion which follows the rapid initial rate, we have in most experiments added only minute amounts of quinine, and do not know certainly whether large amounts can be changed in vitro or not. But although minute chemically the quantities disappearing are very considerable physiologically, if we assume that the living organ can regenerate the active agent or otherwise maintain the reaction at its rapid initial rate. Selecting Experiment A as showing the highest rate of conversion, it is seen that a 0·8 per cent. NaCl extract of 15 grams of guinea-pig's liver converted certainly not less than 6 mgms. of quinine in 90 minutes; this would represent for 1·36 kilos of human

liver a conversion of 11·2 grams (180 grains) of quinine sulphate (73·5 per cent. alkaloid) in twenty-four hours.

In all the experiments quinine was added in solution as the hydrochloride—the quantities stated indicate pure alkaloid. The estimations were made nephelometrically on quinine isolated by the ammonium sulphate process.

EXPERIMENT A. Guinea-pig, dead five minutes. 15 grams of pulped liver were shaken with 100 c.c. of 0·8 per cent. NaCl solution and strained through muslin. 10 mgms. of quinine were added to the strained liquid and samples of this were taken at once and after one and a half hours incubation at 37° C. Strict aseptic precautions were observed throughout, and no bacteria could be discovered microscopically at the end of the experiment.

1.	Boiled at once.	Found 7·7 mgm.	Loss, 23 %
2.	Kept at 37° for 1½ hours.	,, 1·85 mgm.	,, 81 %

EXPERIMENT B. Guinea-pig, dead three hours. 20 per cent. liver emulsion in 0·8 per cent. sodium fluoride solution (as antiseptic) strained through muslin. 10 c.c. mixed at once with quinine. Another 10 c.c. kept at 37° for twenty-four hours before addition of quinine. Both were alkaline to litmus throughout.

		Incubated	Q. found	Loss %
1.	10 c.c. fresh emulsion + 0·5 mgm. Q.	43 hours	0·06	88
2.	10 c.c. stale ,, + ,,	72 ,,	0·38	24

EXPERIMENT C. Guinea-pig, dead nineteen hours. 20 per cent. emulsion in NaF as in B. To two 10 c.c. lots of emulsion added 0·25 mgm. of quinine, one lot in a narrow test-tube, the other in a wide bottle, through which cotton-filtered air was aspirated so as to play on its surface as a strong jet. Both at laboratory temperature.

1.	Found after 20 hours.	0·20 mgm. Q.	Loss, 20 %
2.	Found after 20 hours.	0·13 mgm. Q.	,, 47 %

Whether the beneficial effect of the jet of air was due to mechanical disturbance, removal of prejudicial volatile matter, or to extra supply of oxygen, remains to be seen.

EXPERIMENT D. Same guinea-pig as C. 10 grams of liver-pulp were shaken with 25 c.c. of 50 per cent. alcohol and filtered under suction through a compact layer of kieselguhr sandwiched between two filter papers. The filtrate was rich in

coagulable protein, yellow, and perfectly clear. Two lots each of 2 c.c. + 10 c.c. of 0·8 per cent. NaF were taken. Both were kept at 37° for twenty-two hours after addition of 0·25 mgm. of quinine.

1. Boiled a few seconds before addition of Q. Found 0·245 Q. Loss, 10 %
2. Not boiled. „ 0·15 Q. „ 40 %

EXPERIMENT E. Ox-liver three hours after death. 20 per cent. emulsion in 0·8 per cent. NaF, decanted after ten minutes standing. 10 c.c. of the turbid suspension and 0·5 mgm. of quinine were used in each experiment. All were incubated at 37°C. Even after three weeks no putrefaction was noticeable.

Addition other than Q.	Reaction throughout	Treatment	Hours at 37°	Mgm. Q. found	% Loss
1. 10 cc. of 0·8 % NaCl	Alk.	...	20	0.010	− 98
2. 10 c.c. of 0·8 % NaCl	Alk.	Boiled at once	20	0·043	− 14
3. Nil	Alk.	Filtered through paper	20	0·033	− 94
4. 10 c.c. of 0·4 % HCl	Acid	...	20	0·18	− 64
5. 10 c.c. sat. NaCl Sol.	Alk.	...	70	0·52	− 4
6. 10 c.c. sat. Am_2SO_4	Acid	...	20	0·48	− 4
7. 10 c.c. of 1 % Sod. Arsenite	Alk.	...	70	0·33	− 33
8. ½ c.c. of Chloroform	Alk.	...	90	0·204	− 59
9. ½ c.c. of toluene	Alk.	...	90	0·42	− 14
10. Nil	Alk.	Boiled at once	7 days	0·40	− 20
11. Nil	Alk.	90° for 3 min.	7 days	0·39	− 32
12. Nil	Alk.	40° for 3 min.	7 days	0·18	− 64

EXPERIMENT F. Guinea-pig killed seventy-five minutes after an intraperitoneal dose of 50 mgms. quinine.

The liver was rinsed in dilute salt solution after excision and wiped dry. Strict asepsis throughout.

1. Solid liver as removed. (1 gram) = 0·142 mgm. Q.
2. Solid liver kept at 37° for 18 hours = none.
3. Emulsion in 2 % NaF kept at 37° for 18 hours = none.

The spleen (1 gram containing 0·212 mgm. quinine) and kidney (1 gram = 0·244 mgm. quinine) did not lose the whole of their quinine when similarly treated—the quantities left were not estimated.

EXPERIMENT G. Rabbit's liver. 20 per cent. emulsion in NaF as in E. To 10 c.c. of the strained emulsion 0·5 mgm. of quinine was added.

1. Boiled at once.	Mgm. Q. found 0·45	Difference, 10 %
2. Kept at 37° for 3 hours.	,, ,, 0·20	,, 60 %

EXPERIMENT H. Sheep's blood, defibrinated at once, 10 c.c. mixed with 0·05 mgm. of quinine and kept in at 37° for three days till putrid. Found 0·048 mgm. No appreciable conversion.

The following conclusions may be drawn concerning the conversion of quinine by liver emulsions* in vitro:—

1. After short exposure to a boiling temperature the rate of conversion is very greatly reduced—possibly completely abolished, but more evidence is necessary.

2. In the presence of much NaCl or Am_2SO_4 no quinine is converted.

3. A moderately strong acid reaction does not prevent the change.

4. It occurs in presence of chloroform, NaF, or sodium arsenite, though less rapidly than in their absence. Toluene is very prejudicial.

5. The active agent is extracted by 50 per cent. alcohol, and in such extract can pass through a fine filter; it is probably thermolabile. It 'deteriorates' considerably in a 0·8 per cent. NaF solution (reaction alkaline) during twenty-four hours standing at 37°C.

6. The facts strongly suggest that it is an enzyme, a 'quininase', but further evidence is essential.

QUINOTOXIN

It has been shown by H. C. Biddle (1) that short boilings of an aqueous solution of quinine in the presence of feeble acids, such as acetic acid, results in a yellowing of the solution and the formation of appreciable amounts of quinotoxin. B. F. Howard and O. Chick (6)

* We have learnt since completing this paper that liver emulsions have been shown to destroy Atropin also, (*v.* CLARK (1913)).

have shown that moist crystals of quinine bisulphate are similarly affected at temperatures above 60°C.

For the frequent assumption that the yellowing which results from the action of light on quinine is also attended by formation of quinotoxin, there appears to be no satisfactory evidence.

We have thought it worth while making observations on this alkaloid for two reasons. Firstly, it is conceivable that the quinine which is metabolized in the human body is transformed into quinotoxin as its first downward step, and also that the therapeutic effects of quinine in malaria, or some of its toxic effects, among them possibly blackwater fever, might be connected, directly or indirectly, with quinotoxin, or some metabolite thereof, rather than with quinine itself. Secondly, we have been unable to discover in the literature that any observations have been made of the effects of pure quinotoxin on man, while even on experimental animals they appear to have been remarkably few. Hildebrandt (5) concerned himself mainly with cinchotoxin, and his only statements on the effects of quinotoxin on higher organisms are that it differs from cinchotoxin in that it is less poisonous and does not produce convulsions in warm-blooded animals.

Our attempts to prepare an adequate quantity of the pure substance by Pasteur's and by Biddle's methods proved extremely troublesome, owing to the readiness with which it underwent partial change into a pigmented substance and to the difficulty of applying adequate criteria of purity. We are correspondingly grateful to Messrs. Howards and Sons for kindly presenting us with a liberal sample prepared by Mr. David Howard forty years ago from the uncrystallisable residues of cinchona bark, and shown by him to be identical with Pasteur's compound prepared by melting quinine bisulphate. It gave the Thalleioquin test as delicately as quinine. With Christensen's Herapathite reagent, it gave black oily globules, but very rarely any crystals of Herapathite, and these only in such minute amount as to suggest they were due to a very minute trace of quinine. Evaporated to dryness with excess of dilute hydrochloric acid for three hours on a water bath, as in Elvove's titration method (8), it ' fixed ' exactly the same amount of the acid as did quinine. Titrated by Gordin's method we got variable results which suggested that the method was not applicable

to this alkaloid. From alkaline watery media, it was not so readily extractable by ether as quinine, and for quantitative extraction we found it necessary to saturate the solution with ammonium sulphate. It gives the Tanret turbidity test—with greatly enhanced delicacy (limit of dilution about 1 in 1 million) in solutions saturated with Am_2SO_4. It can be estimated nephelometrically in the same way as quinine, though with less delicacy.

We have found by taking the alkaloid ourselves that with doses up to 200 mgms. (3 grains) once a day by the mouth no appreciable results followed except an increased feeling of 'bien être' such as a small dose of quinine often produces, and a slight looseness of stools. One of us reached 390 mgms. (6 grains) without other effects. One of us vomited forty-five minutes after a dose of 260 mgms. (4 grains), and diarrhoea followed. We are disposed to regard it as an intestinal or gastro-intestinal irritant.

Free quinotoxin was excreted in the urine and recognizable by the Thalleioquin test applied to the extracted alkaloid and by the yellow colour of its solution in HCl. It did not give Herapathite crystals. Whether the whole amount ingested can be recovered from the urine we have not ascertained. But we have found that liver emulsions, even in presence of NaF, attack quinotoxin—a fact which strongly suggests that the living body is as capable of dealing with quinotoxin as it is with quinine.

Experiment I. A 20 per cent. emulsion of ox liver in 0·8 per cent. NaF was prepared, and to 30 c.c. of it was added 1·5 mgm. of quinotoxin dissolved in $1\frac{1}{2}$ c.c. of dilute HCl. After three hours at 37° C. 86 per cent., and after forty-eight hours 92 per cent., had disappeared.

Experiment II. A similar emulsion of guinea-pig's liver was filtered. To 10 c.c. lots of filtrate, 0·25 mgm. of quinotoxin were added. In three hours at 37° C. 64 per cent. had disappeared, in twenty-two hours 68 per cent.

The therapeutic use of quinotoxin in malaria has been investigated by Professors Stephens and Yorke and their colleagues. Their report will be found on p. 217 of the present issue. They show that with a dose of 10 grains on each of two consecutive days, an amount which in the case of quinine would have had very definite effects on the blood parasites, quinotoxin has no appreciable effect.

This result suggests *prima facie* that either it is not a metabolic product of quinine in the human body at all, or that it is quinine itself, or some metabolite along a path which does not include quinotoxin, which is therapeutic in malaria. The question whether quinine is, or is not, metabolized through a quinotoxin stage, and the subsidiary questions bound up with it, nevertheless remain open —*endogenous* quinotoxin may operate very differently.

PARTITION OF QUININE BETWEEN BLOOD AND TISSUES

Any exact knowledge of the relative concentrations of a medicinal alkaloid in the blood and various tissues must have a high degree of physiological and physico-chemical interest. The experiments recorded below, although few in number and furnishing the mere beginnings of such knowledge, bring out several significant points.

The quinine was extracted from all the tissues except brain by the ammonium sulphate method, from brain by the alcohol method, and in both cases estimated nephelometrically. The results with the liver are too low, owing to the rapid modification or destruction of quinine which takes place in this tissue even post-mortem. If such loss occurs in other tissues at all, it occurs less rapidly, and has been, we believe, negligible under the conditions of our experiments, since the control estimates given above were made under very similar conditions.

The only observations found in the literature on quinine in the tissues are those of Giemsa and Schaumann (3) on dogs and guinea-pigs dosed with this drug. Although made by rough comparisons of the results of qualitative tests applied to the quinine extracted from different weights of the tissues investigated, they create a strong presumption that quinine is present at higher concentrations in the tissues (especially in the suprarenal glands) than in the blood.

EXPERIMENT I. Guinea-pig, 400 grams in weight, 0·5 grams of quinine dissolved in 10 c.c. of water and enough HCl to effect its solution was injected into the peritoneal cavity. Forty-five minutes later the already moribund animal was killed. Blood was taken from the heart, defibrinated, and centrifugalised—the quinine-content of the serum and corpuscles (unwashed) was determined

separately. Each of the organs investigated was rinsed free from peritoneal fluid by salt solution and wiped dry before it was weighed.

	Taken	Mgms. Q. found	Mgms. Q. in 100 c.c.
Blood serum	2·6 c.c.	0·1326	5·1
Blood corpuscles	2·2 c.c.	0·033	1·5
			Mgms. Q. in 100 grams
Blood, S.G. 1050	...	...	3·285 calculated
Liver	20·48 grams.	14·85	73
Two kidneys	3·87 ,,	4·025	104
Two suprarenals	0·36 ,,	7·92	2200

The yield of the suprarenals was so immense as to rouse a suspicion that the method of estimation might give excessive results in glands containing adrenalin. We have, however, ascertained that addition of adrenalin to a quinine solution does not affect the accuracy of the estimation, and that pure adrenalin solution or fresh adrenal glands put through the process yield nothing to ether which will give turbidity with Tanret's reagent. We must, therefore, regard the 2·2 per cent. of quinine as having been actually present.

EXPERIMENT II. Guinea-pig, female, weighing 420 grams. Quinine 50 mgms. into peritoneal cavity. No notable symptoms resulted. Killed seventy-five minutes later.

Post-mortem, the spleen and the liver were acutely congested.

	Grams of tissue taken for estimation	Mgms. of Q. found therein	Mgms. of Q. in 100 grams
Blood	4·086	0·0642	1·6
Spleen	3·957	0·84	21·2
Liver	3·015	0·428	14·2
Kidney	1·697	0·414	24·1
Muscle	0·579	0·357	6·2
Suprarenals	0·305	0·492 +	161·0 +

In the suprarenal estimation the quinine extract was lost after the preliminary rough estimation; the result given above is a minimal one.

EXPERIMENT III. Buck rabbit, weighing 2·2 kilos. 10 c.c. of solution containing 400 mgms. of quinine and enough HCl to dissolve it was injected into the auricular vein. The injection was barely complete when the animal unexpectedly died. Blood taken within three minutes of the injection from the superior vena cava was found to contain 40 mgms. of quinine per 100 c.c. Reckoning the blood volume (by Dreyer's formula for rabbits $= \frac{W^{\frac{2}{3}}}{1{\cdot}576}$) as 107 c.c. it would have contained 374 mgms. per 100 c.c. had no quinine left the circulation. It is not very probable, in view of its source, that the blood sample was truly representative of the blood generally, but it is clear that 90 per cent. of its quota of quinine had disappeared within three minutes.

EXPERIMENT IV. Buck rabbit of 1790 grams. Quinine 200 mgms. into peritoneal cavity. No special symptoms except early restlessness and rapid breathing followed by quietness and some prostration. Killed after seventy-five minutes. Tissues removed at once, rinsed, wiped dry and placed in weighed flasks containing acidulated saturated Am_2SO_4 vols.

Tissue.	Mgms. of Q. in 100 grams.
Blood	1·45
Liver	1·26
Kidney	7·0
Muscle	3·1
Suprarenals	5·5
Brain (sample of whole) ...	0·8 (alcohol extraction)
Fat (infra-sternal)	1·24 (alcohol extraction)
Peritoneal fluid	3·12

Fifteen c.c. of peritoneal fluid were obtained (yellow, highly albuminous and turbid, with masses of desquamated endothelium and leucocytes). Of the 200 mgms. of quinine introduced into it certainly less than 1 milligram remained.

The low quinine-content of the adipose tissue may possibly be due to non-attainment of equilibrium with that of the blood-plasma, since this tissue has a poor blood supply. That the partition-coefficient of quinine between fat and blood-plasma or lymph is not strongly, if at all, in favour of fat is however indicated by the rapid effectiveness of intramuscular injections of quinine dissolved in oil. The low finding in brain is, in view of its rich blood supply, not at all likely to be due to non-attainment of equilibrium.

CONCLUSIONS AND COMMENTS

1. The concentration of quinine in normal red blood-corpuscles is very much less than in the surrounding plasma. The difference shown in Experiment I is, owing to inadequate separation of corpuscles from adherent serum, certainly not high enough. The ratio $\frac{\text{Serum Q.}}{\text{Corpuscles Q.}}$ exceeds $\frac{3}{1}$.

2. The concentration of quinine in most of the tissues is very much greater than in blood, *v.* ratio $\frac{\text{Tissue Q.}}{\text{Blood Q.}}$ in the following table:—

	Experiment I		Experiment II		Experiment IV	
	Guinea-pig		Guinea-pig		Rabbit	
Mgms. Q. per 100 grams of body weight	125		12		11·1	
Minutes after dose	45		75		75	
Tissue	Mgms. Q. in 100 grams	Tissue Q. / Blood Q.	Mgms. Q. in 100 grams	Tissue Q. / Blood Q.	Mgms. Q. in 100 grams	Tissue Q. / Blood Q.
Blood	3·285	...	1·6	...	1·45	...
Suprarenal	2200	700	162	100	5·5	3·8
Kidney	104	35	24	15	7·0	4·8
Spleen	...	...	21	13·2	...	...
Liver	(73)	(22)	(14)	(8·7)	(1·26)	(0·87)
Muscle	...	...	6	3·8	3·1	2·1
Adipose	...	...	...	...	1·24	0·8
Brain	...	...	...	...	0·8	0·55

3. With guinea-pigs, enormous concentrations of quinine occur in the suprarenals. The facts recorded by Giemsa and Schaumann strongly suggest that this will prove to be true for dogs also. The absence of any such preponderant accumulation in the rabbit of Experiment IV is no sufficient reason for doubting that it will be found true for rabbits also—this particular rabbit had already disposed of most of the quinine given. Assuming it to be true for man, it may perhaps be correlated with the symptoms of suprarenal inadequacy sometimes observed in malarial patients, more frequently by French physicians.

4. The contrast between the quinine-content of the tissues of the rabbit in Experiment IV and the guinea-pig of Experiment II is a remarkable one. The animals had received intraperitoneally nearly the same dose per 100 grams of body-weight at the same interval before death, and the quinine content of the rabbit's blood was not much less than that of the guinea-pig. As the observations on the peritoneal fluid leave no doubt that the rabbit at least had absorbed all but 1 mgm. of the alkaloid injected, and since not one of its tissues had as high a quinine-content per 100 grams as the dose administered per 100 grams of body-weight, it is certain that a very large proportion of the quinine had already been excreted or metabolized—a very much larger proportion than in the guinea-pig. The contrast may possibly be dependent on the difference of species. Contrasts quite as considerable will, however, be seen later between different men.

QUININE CONTENT OF HUMAN BLOOD AND URINE

The cases investigated are classified according to the dose of quinine and the details of its administration. All were military patients with simple tertian malaria and well habituated to the drug for many months, except where otherwise stated.

The letter T in the columns headed 'malarial parasites' indicates Trophozoites and the letter G Gametes, these facts being kindly supplied to us by Professors Stephens and Yorke and their colleagues. In every case, except where otherwise stated, the blood on the third day of quinine medication was free from parasites.

PATIENT 1, P. A., aet. 20. Intermittent pyrexia (trench-fever ?). Not habituated to quinine.

Dose: First day, 15 grains of quinine sulphate in solution by mouth every three hours, 6 a.m. to 9 p.m. On the two or three occasions when patient vomited the dose, a similar amount was given again. His total intake on this day was therefore probably much more than 90 grains, since absorption is usually rapid.

Second day, 15 grains of quinine hydrochloride by intramuscular injection every three hours from 6 a.m. to 9 p.m.

Total alkaloid ingested > 9070 mgms., total excreted in urine, 1643 mgms. There was diarrhoea and vomiting both days. The faeces of the second day contained more than 100 mgms. quinine.

	Max. Temp.	Mgms. Q. per litre Blood		Mgms. Q. per litre Urine		Ratio $\frac{\text{Urine Q.}}{\text{Blood Q.}}$		Hours excretion after last dose
		1 p.m.	10 p.m.	1 p.m.	10 p.m.	1 p.m.	10 p.m.	
Day 1	101·8°	7·85	8·23	600	1100	76	133	
Day 2	100·6	8·0	6·4	2000	2200	250	390	121
Day 3	100·6	...	...	...	...	...	...	
Day 4	99·6	2·6	...	300	...	115	...	

The excretion of quinine in urine at a concentration three hundred and ninety times as great as that in the blood is probably unique as an instance of the 'secretory power' of the kidney—it is much greater than has been observed with any of the normal constituents of urine which we have found on record.

GROUP A, aet. 20 to 36 years.

Dose: 15 grains of quinine sulphate (73½ per cent. alkaloid) in solution, at 6, 9, 12 a.m. and 3, 6, 9 p.m. on each of two successive days.

The first six cases of the group are strictly comparable, if we treat as negligible the small losses of quinine in the stools of 2 and 4.

In Case 8, even on the third day at 1 p.m. (twenty-seven hours after the last dose) the amount of quinine per litre of blood was still considerable—8·3 mgms. Malarial parasites could no longer be found. It is remarkable, in view of the very much larger amount of quinine in his blood, that the parasites persisted longer than in most of the other patients—possibly it is not the quinine which is

Patient	Max. Temp.		Mgm. Q. per litre Blood				Malarial parasites in blood		Mgm. Q. in urine	% Q. excreted	Hours excretion after last dose
			1st day		2nd day.						
	1st day	2nd day	1 p.m.	10 p.m.	1 p.m.	10 p.m.	1st day	2nd day			
2 HU	102·8°	103·2	3·8	4·5	2·2	2·0	T.	O.	487	6·8	154
4 MO	98	98	3·3	...	1·0	2·2	T.	O.	...	...	...
5 PO	98·4	97°	1·6	2·0	1·0	4·4	T.G.	O.	...	...	...
6 MI	100·6	99	5·5	5·0	2·5	5·0	T.G.	O.	...	...	...
7 RY	97	97	3·57	3·0	3·3	13·3	T.	O.	574	6·7	111
8 WA	99·4	104	16·6	16·6	16·6	16.6	T.G.	T.	913	10·6	187
9 SH	102	98	11·1	4·5	2·1	6·6	T.G.	O.	203	(2·4+)	128
10 DO	100	100	1·0	5·5	6·6	10·0	T.G.	T.G.	648	(7·5+)	133

present but the quinine which has disappeared (i.e. one of its metabolites) which is obnoxious to the parasites.

In Case 2 the quinine of each specimen of the urine passed after 6 a.m. on the first day until 7.15 p.m. on the fourth day, when quinine ceased to be excreted, was estimated separately. Graph I, p. 254, shows the results obtained. Getting, by interpolation the concentration of the urine at the times when the blood samples were taken, the $\frac{\text{Urine Q.}}{\text{Blood Q.}}$ ratio is obtained.

Case 2.	Mgm. Q. per litre urine	$\frac{\text{Urine Q.}}{\text{Blood Q.}}$
1st day 1 p.m.	95	25
,, 10 p.m.	105	23
2nd day 1 p.m.	170	80
,, 10 p.m.	130	75

In Cases 9 and 10 the losses by vomiting are unknown and may have been considerable.

Attention is called to the following points:—

1. The great differences of concentration of blood-quinine in the different cases—presumably due to differences either in the rate of metabolism of quinine or in the rate of its excretion.

2. The association of a high quinine-content of blood (> 11 mgms. per litre) with symptoms of quinine intoxication. In Case 8, W. A., with 16·6 mgms., these were so severe throughout that administration of stimulants was often necessary. This association was specially striking in Case 9, with 11 mgms. at 1 p.m. and only 4·5 mgms. at 10 p.m. From 9 a.m. to 6 p.m. the patient was suffering severely with headache, prostration, nausea, rapid pulse, and buzzing in the ears. After this his condition rapidly improved, and next day he was quite comfortable. Case 7 reached 13·3 mgms. per litre of blood only at the end of the period, and possibly maintained this only for a short time, but unfortunately we have no record of his symptoms.

3. The association of the persistently high quinine-content of the blood in Case 8 with marked urobilinuria. In no other case, except with actual blackwater fever, has there been any increase of urobilin, although many have been feverish. Since urobilinuria is a common precursor of the haemoglobinuria of 'blackwater,' its appearance in this case is suggestive of some increased haemolysis due directly or indirectly to quinine.

PATIENT 13, K. N., aet. 35.

Dose: First day, 30 grains quinine sulphate at 10 a.m. and 15 grains at 1, 4 and 7 p.m. Total 75 grains.

Second day, 15 grains every three hours, 6 a.m. to 9 p.m. Total 90 grains. Vomited at 11 p.m. on first day. Total quinine 7880 mgms. alkaloid less amount lost in vomit.

No diarrhoea and only one stool (containing a negligible trace of quinine).

Max. Temp.		Mgm. quinine per litre of blood.				Malarial parasites in blood	
		1st day		2nd day			
1st day	2nd day	1 p.m.	10 p.m.	1 p.m.	10 p.m.	1st day	2nd day
100·2°	99·2°	1·6	2·0	6·6	7·2	T.G.	T.G.

GROUP B.

Dose: 15 grains quinine sulphate in solution by mouth every four hours, beginning at 10 a.m. on first day. No vomiting or diarrhoea.

Total quinine sulphate before 1st blood sample 15 grains.
" " " " 2nd " " 45 "
" " " " 3rd " " 105 "
" " " " 4th " " 135 "

Patient	Max Temp.		Mgm. Q. per litre Blood				Malarial parasites in blood	
			1st day		2nd day			
	1st day	2nd day	1 p.m.	10 p.m.	1 p.m.	10 p.m.	1st day	2nd day
11 JOI	98·4°	98°	0·5	...	1·0	4·0	T.G.	O.
12 OW	104	98·4	0·5	0·6	4·8	9·9	T.G.	T.G.

Case 12, O. W., is the only one, except 17, K. N., whose blood still contained malarial plasmodia (*P. vivax*) on the third day: they were not found on the fourth day.

GROUP C. INTRAMUSCULAR INJECTIONS.

Both cases had received 30 grains of quinine hydrochloride by mouth and 30 grains by intramuscular injection on each of the twelve days preceding. On the first day of our observations both had received at 4 and 7 a.m. 15 grains quinine salt by mouth, and (the last dose) at 9.30 a.m. 30 grains quinine salt intramuscularly. No vomiting or diarrhoea.

Patient	Age	Max. Temp.		Mgm. Q. per litre Blood		Mgm. Q. per litre Urine		Ratio $\frac{\text{Urine Q.}}{\text{Blood Q.}}$		Hours duration of excretion after last dose
		1st day	2nd day	3 hours after last dose	27 hours after last dose	3 hours after last dose	27 hours after last dose	3 hours after last dose	27 hours after last dose	
14 DA	21	98·4°	98·4°	3·23	1·97	337	255	104	129	115
15 JOW	29	98·4	99·2	1·98	1·42	168	79	85	56	107

Malarial plasmodia were absent on both days.

GROUP D. SMALL DAILY DOSES.

5 grains of quinine sulphate (238 mgms. alkaloid) orally, in solution, every morning, except Sundays, at 9 a.m. during the three weeks preceding the Thursday on which the blood and urine were sampled.

Patient	Mgms. Q. per litre, blood, 3 hours after last dose	Mgms. Q. per litre of Urine	Urine Q. / Blood Q.
17 KN	2·3 (12.30 p.m.)	73·3 (1.15 p.m.)	31·8
18 TO	1·6 ,,	22 (12.30 p.m.)	13·6
19 BU	1·9 ,,	18·65 (1.15 p.m.)	9·8

In Case 17, K. N., malarial parasites were present in the blood every day; they were absent in Case 18, T. U., and Case 19, B. U., although the quinine content of the blood was decidedly lower.

GROUP E. INTRAVENOUS INJECTIONS.

A solution of quinine hydrochloride (81·6 per cent. alkaloid) was injected into the median basilic vein of one arm, and a sample of blood was then as quickly as possible (certainly within one minute) withdrawn from that of the other arm.

	Mgm. Q. alkaloid injected	Mgms. Q. if distributed equally through 3 litres of blood	Mgms. Q. per kilo injected	Found Mgm. Q. per litre blood (S.G. 10607)	% Q. disappeared from blood
20	530	177	7·5	44·2	77
21	530	177	7·5	23·6	87
22	480	160	6·8	16·6 and 3 hours later 4·42	90

In Case 22 a sample of urine 25 c.c. passed fifty minutes after injection of the quinine contained 240 mgms. per litre. Assuming a linear drop in concentration of quinine in the blood, although it would doubtless be much more rapid, this would give a minimum $\frac{\text{Urine Q.}}{\text{Blood Q.}}$ ratio of $\frac{240}{13{\cdot}2} = 18$, or a maximum of $\frac{240}{4{\cdot}42} = 54$.

The excretion time was sixty-six hours, as judged by the fact that the urine then ceased to give the Herapath test.

Total quinine excreted in 2400 c.c. urine was 17·9 mgms. Percentage excreted $\frac{179}{480} \times 100 = 37{\cdot}3$.

CASE 16, M. X., aet. 29. Malignant tertian (*Plasmodium falciparum*).

First attack of blackwater fever.

Date	Max. Temp.	15 grain dose of sulphate Q. by mouth	Mgm. Q. in litre blood	Malarial parasites	Mgm. Q. in litre urine	Urine Q. / Blood Q.
12.4.18	100 F	At 2, 6, and 10 p.m.	...	T.G.	...	...
13.4.18	103	At 9 a.m. (vomited soon after)	13·3 (at 12.30 p.m.)	T.G.	12·5 (at 12.30 p.m.)	0·94
14.4.18	98·4	Nil.	...	...	...	...

Haemoglobinuria began at 4 p.m. on 12th April, two hours after the last dose, and lasted thirty-two and a half hours till 12.30 p.m. on the 13th April.

The quinine-content of the blood on the 13th April is a very large one for such a dose of quinine at such an interval from the dose, especially as the last dose was vomited soon after it was given.

The ratio $\frac{\text{Urine Q.}}{\text{Blood Q.}}$ is unique in our observations. It is the only one not showing quinine in the urine at considerably greater concentration than in the blood. It is evident that the power of the kidney to excrete quinine was greatly diminished.

Second attack of blackwater fever.

Date	Max. Temp.	15 grain doses of Q. sulphate by mouth	Malarial parasites	Vomited	Mgm. Q. in litre blood	Mgm. Q. in litre urine	Urine Q. / Blood Q.
24.5.18	...	At 10, 2 and 6 p.m.	...	0	...	...	...
25.5.18	104°	At 10 a.m. and 2 p.m.	...	6 times	...	...	...
26.5.18	98	Nil.	...	...	1·19 at 1 p.m.	18·68	15·6

Haemoglobinuria began at 5.45 p.m. on the 25th May (three and three-quarter hours after the last dose of quinine), and lasted till 10.30 p.m. of the same day—altogether four and three-quarter hours. Urobilinuria lasted twenty-one hours. As the urobilin was disappearing an abnormal pigment not further investigated made its appearance and coloured the precipitate produced in Schlesinger's urobilin test (addition of an equal volume of 10 per cent. alcoholic zinc acetate solution to the urine) a salmon pink—this phase lasted thirty-two hours. Acetone and diacetic acid were absent.

The points of special interest in these two attacks are the low $\frac{\text{Urine Q.}}{\text{Blood Q.}}$ ratio during the first attack, and the fact that even on the day after a second milder attack, this ratio, although showing a considerable return of the secretory power of the kidney, is still much less than it would be in any normal patient.

It is an interesting question whether the defect in the secretory power of the kidney for quinine was a primary condition, and the large amount of quinine in the blood resulting therefrom the cause of the haemolysis, or whether the haemolysis occurred first and by diminishing the excretory functions of the kidneys for quinine was ultimately responsible for the high quinine-content of the blood. That the functions of the kidneys in secreting some at least of the other constituents of urine were not seriously impaired during the 'blackwater' period is shown by the high secretion-rate of 7·6 c.c. per minute which was observed.

The secretory activity of this patient's kidneys was also investigated (by the phenol-red test) in a non-haemoglobinuric period intervening between the two attacks of blackwater fever. 72 per cent. of the phenol red administered was found in the urine in the first two hours—evidence, so far as any one test can supply it, of good renal function.

The rate of excretion of quinine was also investigated during this normal period. On April 26th, at 2 p.m., a solution of 45 grains of quinine sulphate (2150 mgms. alkaline) was administered orally.

After fifty-five hours the Herapath test was no longer obtainable from 40 c.c. of urine.

The total quinine excreted in the fifty-eight hours was 487 mgms. = 22 per cent. of the quinine administered.

Graph II, p. 255, shows the Rates of excretion of Urine and of Quinine and the concentration of quinine in the excreted urine. Apart from the initial rise in quinine concentration, it will be seen that almost every increase in the rate of excretion of urine is attended by a reduction in the concentration of the quinine in the urine.

QUININE IN FAECES

There is a general consensus of opinion that after the administration of solutions of quinine in moderate doses the amount which is excreted with the faeces is so small as to be negligible.

One of us after a dose of 30 grains of the hydrochloride on each of two consecutive days was unable to detect even a trace in his faeces by a method which would have yielded a positive result with as little as 0·1 mgm. of quinine in 10 grams of faeces.

Of the men taking 90 grains of the sulphate (4300 mgms. alkaloid) on each of two consecutive days, only those rendered diarrhoeic thereby produced faeces in which quinine could be detected in more than traces.

In Case 2, Group A, the 218 grams of semi-fluid faeces of the first two days yielded only 24 mgms. of quinine as estimated by the 'minimal' method described earlier—and could hardly have contained more than 50 mgms. (= 0·6 per cent. of the 8600 administered). In Case 1, where many fluid stools were passed, the amount was much greater—the faeces of the first day were lost, but those of the second day contained at least 100 mgms. quinine—probably at most 200 mgms.

In Cases 4, 11 and 13, with normal stools the amount of quinine was so small that it was impossible to get crystals of Herapathite from them. When the stools have not been 'loose,' we have therefore felt justified in assuming that excretion by the faeces was negligible.

INTERVAL BETWEEN DOSE OF QUININE AND APPEARANCE IN URINE

Unless catheterization be resorted to, only 'maximal results' can be obtained. Examining urine passed naturally by eighteen healthy

men each of whom had taken a solution of 0·33 gram (5 grains) of quinine sulphate on an empty stomach, the times obtained were:—

8	minutes	1 case.
10	„	1 „
18	„	11 cases.

With five men taking 15 grains of quinine sulphate in solution on an empty stomach, quinine appeared in the urine passed after the following intervals:—

9	minutes	1 case.
10	„	1 „
12	„	1 „
14	„	2 cases.

There can therefore be no doubt that quinine administered in solution as the sulphate to fasting men begins to be absorbed, and also to be excreted in the urine, with considerable rapidity.

DURATION OF EXCRETION

The number of hours after the last dose of quinine during which it was possible to obtain Herapathite crystals from the ether extract of 40 c.c. of urine has been determined in some of the cases. The end-point would generally correspond to a quinine-content of less than 0·025 mgm., in the 40 c.c. of urine. It is difficult at present to attach much significance to the results.

Single dose taken fasting:—

Mgms. of quinine alkaloid taken orally in solution	Secretion period of last sample of urine containing quinine
100	38—47 hours
100	34—41 „
238 (5 grains of Q. sulphate)	39—45 „
238 (5 grains of Q. sulphate)	52—64 „
238 (8 cases out of 10)	48—60 „
238 (2 cases out of 10)	60—72 „
480 (intravenous)	60—66 „
500	40—41 „
2150 Case 16 MX	50—55 „

It appears probable for single doses taken by the mouth that the excretion-time, although differing greatly with different men, is much the same whether the dose be large or small.

Data of the excretion-period after the last dose of a long series have been recorded with the grouped cases. The longest period was seven and a half days in Case 8, the patient with the highest concentration of quinine in his blood.

THE CONCENTRATION OF QUININE IN URINE AND ITS RATIO TO THAT IN BLOOD

In Cases 1, 2, 14, 16 and 22, the maximal concentration expressed as mgms. quinine per litre of urine have been 3000, 200, 255, 219 and 240 respectively. Our facts do not support Nierenstein's (7) statement that 'there is a tendency for the excretion of the quinine passed to reach a concentration of 7 to 11 grains (450 to 650 mgms.) per litre of urine.'

In every case, except 16, during an attack of blackwater fever, the concentration in urine has been, *at all stages* investigated, many times greater than in the contemporaneous blood, the $\frac{\text{Urine Q.}}{\text{Blood Q.}}$ ratio ranging from 9 to 390. Even with so small a dose as 238 mgms. quinine (5 grains of quinine sulphate as ordinarily prescribed), a ratio of 31 was found three and a half hours after the dose (Case 17).

PERCENTAGE OF ADMINISTERED QUININE FOUND IN BLOOD

The maximum percentage was found in Case 17 on the small daily dose of 238 mgms. quinine. Assuming the volume of blood to have been three litres, there was $\frac{3 \times 2{\cdot}3 \times 100}{238} = 2{\cdot}9$ per cent. of the quinine ingested.

THE 'ACME' OF EXCRETION

According to Hartmann and Zila (4), this occurs between four and eight hours after oral administration of a single dose of quinine (quantity not known to us). In Case 16, M. X., after a dose of 2150 mgms., during a non-haemoglobinuric period, the maximum excretion rate (0·55 mgm. quinine per minute) as also the maximum

concentration of quinine (219 mgms. per litre), was found in the urine of the period twenty-three to twenty-five hours. Whether a late 'acme' is general with a large dose or was a peculiarity associated with the liability of the patient to blackwater fever remains uncertain.

PERCENTAGE OF QUININE EXCRETED

A study of the literature shows that with men taking moderate doses of a soluble salt of quinine, whether by mouth or by intravenous or intramuscular injection, the percentage of the quinine which leaves the body differs greatly in different men, but has never been found less than 23 or greater than 66.

This has been true also of the few cases in which we have ourselves determined the percentage after single doses. We should add, however, that Hartmann and Zila (2) have recently recorded after oral administration a total excretion of 15 to 35 per cent.

With 238 mgms. orally ... we found 23 per cent. (E. W.).
„ 480 „ intravenously „ „ 37 per cent. (Case 22).
„ 2150 „ orally „ „ 24 per cent. (Case 16).

But with the men of Group A taking very large doses (90 grains a day on each of two successive days) the percentage excreted was unprecedently low.

Case 2, H. U., 6·8 per cent. = 93 per cent. metabolized.
„ 7, R. Y., 6·7 per cent. = 93 per cent. „
„ 8, W. A., 10·6 per cent. = 89 per cent. „

For a satisfactory explanation of this paradox, a better all-round knowledge of the fate of quinine inside the body and of the rate of its absorption from the alimentary canal under normal and abnormal conditions is essential. The explanation is likely to have much clinical interest.

DISCUSSION

1. It is probable that the diminished percentage excretion of quinine by the patients of Group A is bound up with the equally remarkable diminution in the amount of quinine found in the blood of most of them at 1 p.m. on the second day of quinine medication, and also at 10 p.m. in the two cases with abnormal intestinal conditions (Cases 2 and 4).

It is for the light it throws on this question, and also on the functioning of the kidney, that we have thought it desirable to publish Graph I relating to Case 2, the only one of Group A in which the quinine of each sample of urine was estimated separately. That the patients (Cases 2, 4, 5, 6, 7 and 8) did actually ingest the quinine and retain it there is no doubt. That none of the urine was lost we have full confidence.

The quinine of the third, fourth and fifth days' faeces was not estimated, but as these were not loose they were assumed to contain only negligible amounts. There was no stool on the first day, but that of the second day (late evening) was semi-fluid and contained at most 50 mgms. We have very little doubt that the total loss of quinine in the combined faeces of the whole five days was well below 100 mgms.

The total output of quinine in the urine, calculated by summing up the nephelometric estimations of 5 c.c. of each individual sample, was 476 mgms. A gravimetric estimation by the Iodine Potass-Iodide precipitation method described by two of us (1), was made on a representative quarter of the total 5·6 litres of urine of the four days (obtained by mixing together a quarter of each of the seventeen specimens of urine)—found for total urine 484 mgms. quinine, a very satisfactory confirmation of the accuracy of the nephelometric estimations. Allowing 100 mgms. for the faeces, the total quinine leaving the body, expressed as a percentage of the quinine ingested, was $\frac{584}{8600} \times 100 = 6{\cdot}78$ per cent.

The figures given below show the quinine excretion in the urine in successive twelve-hour periods:—

Case 2	Mgm. Q. Ingested	Mgm. Q. excreted in urine
0—12 hours	2866 (60 grains Q. Sulph.)	197
12—24 ,,	1433 (30 grains Q. Sulph.)	116
24—36 ,,	2866 (60 grains Q. Sulph.)	112·6
36—48 ,,	1433 (30 grains Q. Sulph.)	22·8
48—60 ,,	Nil.	17·4
60—72 ,,	Nil.	7·0

It will be seen that although in the first twenty-four hours 313 mgms. of quinine was excreted, in the second twenty-four hours not more than 185 mgms. (135 mgms. in urine + 50 mgms. in faeces) left the body, and in the third twenty-four hours not more than 77 mgms. (27 + 50 mgms. in faeces).

To explain this falling off on the second day, it is necessary to assume that either a very much smaller proportion of the quinine ingested was absorbed from the intestine, the unabsorbed residue being partly expelled with the faeces and partly altered by bacteriolytic or other agencies; or as an alternative that absorption was quantitatively normal but a very much larger proportion of the absorbed quinine was metabolized.

Coming to the detailed curves of quinine excretion, attention is called to the interesting fact that, except for the two initial and final samples of urine where quinine was just beginning or ceasing to be available for excretion, every increase or decrease of the rate of excretion of urine was *without exception* accompanied by an increase or decrease of the rate of excretion of quinine (*v.* Graph I), and that very frequently the more rapidly the urine was being secreted the greater was the concentration of the quinine in it.

In Graph II, presenting the results obtained with the patient (Case 16, M. X.) who was subject to blackwater fever, it will be seen that although nearly every increase in the rate of secretion of urine was accompanied by an increased rate of excretion of quinine, the quinine was excreted at a *smaller* concentration.

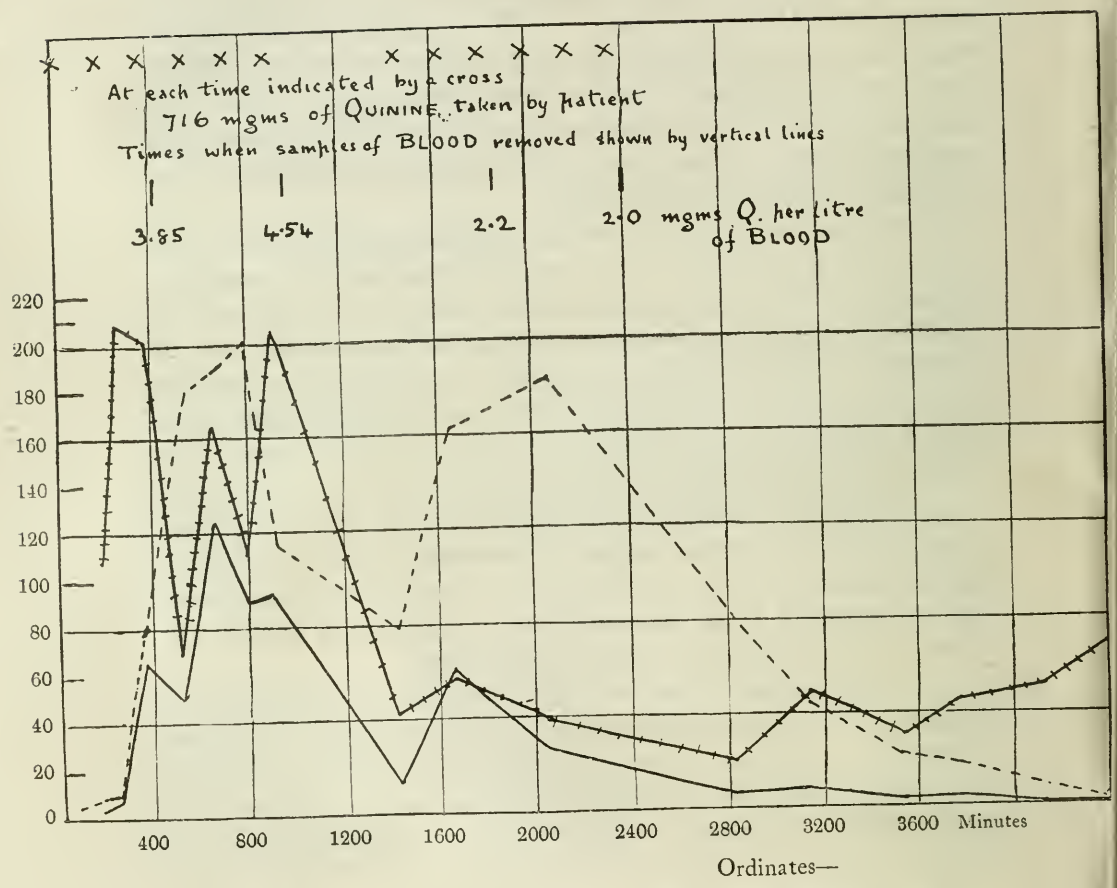
2. The quinine-content of the blood of the various cases subjected to precisely similar medication shows very great individual differences. Compare in Group A, Case 5, with 1·6, 2·0, 1·0 and 4·4 mgms. quinine per litre in the successive samples of blood, and Case 8, with 16·6 mgms. quinine per litre in each sample, even the very first.

The various cases differ greatly, not only in the maximal concentrations attained but also in the rapidity with which the maximum is reached. Further, as has already been pointed out, most of them have less quinine in the third sample than in the first.

These differences in the amount of quinine in the blood are doubtless accompanied by (more or less proportional ?) differences in the concentration of the quinine in the tissues.

GRAPH I.

CASE 2. HU.

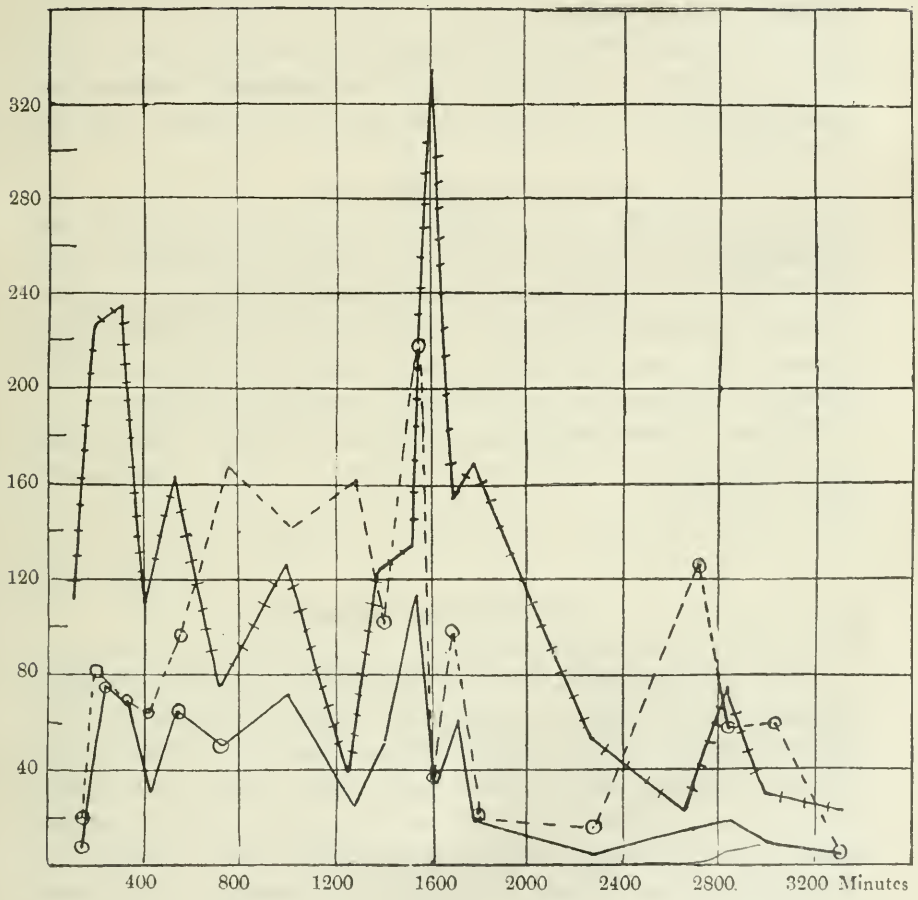


Ordinates—

———	Urine secretion rate.	C.c. urine in 50 minutes.
———	Quinine excretion rate.	Mgs. quinine in 200 minutes.
- - - - -	Concentration of quinine.	Mgms. quinine in 1 litre of urine.

GRAPH II.

CASE 16. MX. 2150 mgms. quinine by mouth, as quinine sulphate.



Ordinates—

Urine secretion rate.	C.c. of urine in 50 minutes.
Quinine excretion rate.	Mgms. of quinine in 200 minutes.
Concentration of quinine.	Mgms. of quinine per litre of urine.

3. Comparing men taking different doses by mouth, it should be noted that one of the patients taking 5 grains of quinine sulphate once each day (Case 17, Group D) attained as high a level of quinine concentrations in his blood three and a half hours after the dose as many of the patients of Group A after they had taken 90 grains (15 grains every three hours).

4. A quinine-content of 2·3 mgms. per litre of blood was in Group D, Case 17, compatible with the continued existence of malarial plasmodia in the blood day after day throughout a period of three weeks.

5. As we are informed that relapse occurred eventually in every one of the malarial cases in which the quinine of the blood was estimated, it is clear that even 16·6 mgms. quinine per litre maintained at a steady level for at least thirty-three hours has not sufficed to prevent a relapse, and therefore that no concentration of quinine in the blood at all likely to be compatible with the continued existence of the host can be counted on to effect a radical cure.

Whether a certain minimum percentage of quinine in the blood may be necessary to cut short a particular attack of malaria is another question.

CONCLUDING REMARKS

To the facts recorded concerning quinotoxin we would add that we have seen no reason to suspect its presence in the urine of patients taking quinine—the quinine obtained by the Iodine KI process has always been colourless even though dried at 120° C., or at worst of a very pale lemon tint, and it has given Gordin titration values in close agreement with the gravimetric results. Nevertheless, the possibility that a trace of quinotoxin is present in some cases should be kept in view, although its detection would be by no means easy. As a hypothetical metabolite of quinine it might quite well have a fugitive existence as it arises inside the body.

Further investigation of the changes produced in quinine by liver-extracts appears to us urgently desirable as the most direct means of ascertaining the fate of the 23 to 93 per cent. of the ingested quinine which is metabolized in the body. Our observations

hitherto have had a merely preliminary character. A full knowledge of the metabolism of quinine may well be essential for any adequate explanation of its therapeutic effects and failures.

We are much indebted to Professor Stephens and Professor Yorke for the many facilities they have afforded us in obtaining specimens from malarial patients, and to Professor Yorke for much kindness in making the various intraperitoneal and intravenous injections referred to in the paper.

RÉSUMÉ

1. Delicate methods are described for the estimation and detection of quinine in animal tissues and liquids.

2. Quinine does not normally suffer change in putrifying urine or faeces.

3. Quinine introduced into an animal in large doses accumulates in most of the tissues at very much higher concentrations than in the blood.

4. Of the quinine present in the blood, more than three-fourths is in the serum (plasma ?). Normal red corpuscles take up very little quinine.

5. After intraperitoneal injections the suprarenal glands take up quinine at much higher concentration than any other tissue examined; the kidneys probably come next in the series.

6. The healthy human kidney excretes quinine at much higher concentration than that at which it is present in the contemporaneous blood. During an attack of blackwater fever it appears to lose this power.

7. The liver of rabbits, guinea-pigs and oxen rapidly attacks quinine post-mortem and presumably during life. The properties of the active agent suggest that it is an enzyme. The product or products presumably represent normal metabolites of quinine in the living body.

8. Experiments directed to ascertain whether quinotoxin is a normal metabolite have shown that (*a*) it is attacked by liver extracts; (*b*) when ingested by mouth it produces alimentary disturbances, but some is absorbed and some at least is excreted unchanged in urine;

(*c*) any anti-malarial action which it may exert is so slight in comparison with that of quinine as to be negligible.

9. A given dose of quinine gives rise in different men to very different amounts of quinine in the blood.

10. The excretion-period of quinine by the urine differs greatly in different men—ranging from forty-one hours (after a single dose by mouth) to seven and a half days (after the last of a succession of large doses).

11. About 90 per cent. of the quinine injected intravenously disappears from the blood within one minute.

12. There is a striking association between symptoms of quinine intoxication and high concentrations of quinine in the blood.

13. When quinine is administered in a succession of large doses, an abnormally large proportion (from 90 to 93 per cent. of that ingested) is metabolised.

14. Quinine may fail to effect a radical cure of malaria even when it has reached, and maintained for some time, a concentration in the blood so high as to be barely tolerable to the patient.

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